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(54) Title: USE OF VANILLOID RECEPTOR ANTAGONISTS FOR THE TREATMENT OF UROLOGICAL DISORDER

(57) Abstract: The present invention relates to methods for treating urological disorders. More particularly, this invention concerns the use of a vanilloid receptor (VR1) antagonist for the prophylaxis and treatment of urinary incontinence and overactive bladder.

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USE OF VANILLOID RECEPTOR ANTAGONISTS FOR THE TREATMENT OF UROLOGICAL DISORDER

DETAILED DESCRIPTION OF INVENTION

TECHNICAL FIELD

The present invention relates to methods and pharmaceutical compositions for treating urological disorders. More particularly, this invention concerns the use of a vanilloid receptor (VR1) antagonist for the treatment of urinary incontinence and overactive bladder.

BACKGROUND ART

Vanilloid compounds are characterized by the presence of vanillyl group or a functionally equivalent group. Examples of several vanilloid compounds or vanilloid receptor modulators are vanillin (4-hydroxy-3-methoxy-benzaldehyde), guaiacol (2-methoxy-phenol), zingerone (4-/4-hydroxy-3-methoxyphenyl/-2-butanon), eugenol (2-methoxy4-/2-propenyl/phenol), and capsaicin (8-methy-N-vanillyl-6-nonene-amide).

Among others, capsaicin, the main pungent ingredient in "hot" chili peppers desensitizes sensory neurons or C-fiber afferent neurons. Capsaicin and its analogues, such as resiniferatoxin(RTX), are shown to be effective in the treatment of urological disorder e.g., urinary incontinence and overactive bladder, through the desensitization of C-fiber afferents [(Chancellor, M. B. and de Groat, W. C., The Journal of Urology, 162, 3-11, 1999) and (Andersson, K-E et al, BJU International, 84, 923-947, 1999)].

Vanilloid receptor is a specific neuronal membrane recognition site for capsaicin. It is expressed almost exclusively by primary sensory neurons involved in nociception and neurogenic inflammation. Capsaicin functions as a specific agonist of vanilloid

receptors (VR1), which is a functional subtype of vanilloid receptor and predominantly expressed in cell bodies and nerve endings of dorsal root ganglion (DRG) neurons or afferent sensory fibers including C-fiber (Tominaga M. et al, Neuron. 21: 531-543, 1998). The VR1 receptor was recently cloned (Caterina, M.J., et al, Nature, 389 (6653), 816-824 (1997)) and identified as a nonselective cation channel with six transmembrane domains that is structurally related to the TRP (transient receptor potential) channel family. Binding of capsaicin to VR1 opens the ion channel and allows sodium, calcium and possibly potassium ions to flow down their concentration gradients, causing initial depolarization and release of neurotransmitters from the nerve terminals of C-fiber afferents. That results in incontinence and overactive bladder. Repeated or prolonged exposure of VR1 receptor to capsaicin or RTX, however, rapidly desensitizes them. This acute desensitization of VR1 may be caused by the downregulation and/or internalization of VR1 triggered by the agonists [(Lower Urinary Tract Symptoms. Eds by Kosuke Yasuda et al., Miwa shoten Press, 2000) and (Goso, C; Piovacari, G and Szallasi, A, Neuroscience Letters, 162 197-200, 1993)]. This "acute" level of desensitization does not last long, and VR1 recovers its response to capsaicin or RTX in hours. Different from this, exposure of C-fibers to high concentration of capsaicin or RTX desensitizes neurons themselves. This may be due to the depletion of neurotransmitter by the strong stimulation of VR1 with agonists (Avelino A and Cruz F, Auton Neurosci; 86 (1-2): 37-46, 2000). Another possible mechanism of the long term desensitization may be the decrease of the transport of nerve growth factor (NGF) to the cell body by affecting the axonal signal transmission, that in turn leads to depletion of neuropeptides, thus paralyzing the function of C-fiber (Lower Urinary Tract Symptoms. Eds by Kosuke Yasuda et al., Miwa shoten Press, 2000). In addition, the specific neurotoxic action of capsaicin and RTX may induce intracellular accumulation of ions leading to osmotic changes and activation of proteolytic enzyme processes to finally deteriorate C-fiber.

Although the mechanism in which capsaicin and RTX cause the desensitization of C-fiber afferents is complicated and yet not fully understood, capsaicin and RTX are

thought to act as "VR1 superagonists", whose strong signals through VR1 receptors result in desensitization of C-fiber afferent nerves themselves and consequent low levels of the neurotransmitter release (cell level suppression).

Although it is not decisive, anandamide and several other lipid metabolites are speculated as endogeneous agonists for VR1 [(Zygmunt, PM. et al, Nature 1999, 400, 452-456) and (Hwang, SW. et al, Proc Natl Acad Sci USA 2000, 97, 6155-6160)]. These "endogeneuos" agonists would be synthesised and could be the cause of incontinence or overactive bladder. However, C-fiber can also be activated by various other stimuli such as ATP for P2X3 and P2Y, prostaglandin E2 for EP receptors, prostaglandin I2 for IP receptor, and nerve growth factor for TrkA. All of these stimuli on C-fiber are produced in stretched- or diseased bladder. Therefore, it is uncertain that VR1 antagonists, by just blocking the channel activity of VR1 at molecular level, would show similar efficacy achieved with high dose of capsaicin or RTX on incontinence and overactive bladder.

Capsazepine, a capsaicin analog, has been reported to be a capsaicin receptor antagonist. (Urban L. et al. Neurosci. Lett. (1991), 134(1), 9-11).

International Applications, publication numbers WO01/85158, WO02/072536, WO02/08221, WO02/090326, WO02/16317, WO02/16318 and WO02/16319 each disclose certain vanilloid receptor antagonists and their use for the treatment of diseases associated with the activity of vanilloid receptor.

SUMMARY OF THE INVENTION

This invention is to provide a use of a VR1 antagonist in the preparation of a medicament for treating or preventing urological disorder.

This invention is also to provide a pharmaceutical composition which includes a VR1 antagonist for treatment and/or prophylaxis urological disorder.

Further this invention is to provide a method for treating urological disorder in a mammal, preferably in a human, which method comprises administering to said mammal a therapeutically effective amount of a VR1 antagonist.

In certain embodiments, the VR1 antagonist is selected from the group consisting of N-[4-chloro-3-(trifluoromethyl)phenyl]-N'-(2,4-dibromo-7-hydroxy-1-naphthyl)urea; N-[4-chloro-3-(trifluoromethyl)phenyl]-N'-(2,4-dichloro-7-hydroxy-1-naphthyl)urea and N-(4-bromo-2-chloro-7-hydroxy-1-naphthyl)-N'-[4-chloro-3-(trifluoromethyl)phenyl]urea,

or pharmaceutically acceptable salts thereof.

In another embodiments, the VR1 antagonist is an antibody immunoreactive with VR1.

EMBODIMENT OF THE INVENTION

The present invention provides a pharmaceutical composition which includes a VR1 antagonist for treatment and/or prophylaxis of urological disorder and a use of a VR1 antagonist in the preparation of a medicament for treating or preventing urological disorder. "Urological disorder" used herein includes all kind of urological diseases and dysfunction. Typical example of the urological disorder is overactive bladder, lower urinary tract symptoms and urinary incontinence, which encompasses detrusor hyper-reflexia, detrusor instability, urgent micturition, micturition of increased frequency, urge urinary incontinence, mix urinary incontinence and the like.

"VR1 antagonists" include species that will bind VR1 and species that will interfere with the binding of VR1 to its ligands, vanilloid and other natural compounds. Antagonists that bind VR1 include, without limitation, monoclonal or polyclonal

antibodies and fragments thereof, chimeric antibodies and fragment thereof and certain groups of organic small molecules. Antagonists that interfere with VR1 binding include, without limitation, monoclonal or polyclonal antibodies and fragments thereof, chimeric antibodies and fragment thereof and certain groups of organic small molecules.

VR1 antagonists can be produced by methods well known to those skilled in the art. For example, monoclonal VR1 antibodies can be produced by generation of antibody-producing hybridomas in accordance with known methods.

VR1 used in the present invention can be any form of VR1, so long as that form of VR1 is capable of binding its ligands.

Typical salts of an organic small molecule include salts prepared by reaction of the compounds with a mineral or organic acid, or an organic or inorganic base. Such salts are known as acid addition and base addition salts, respectively.

Acids to form acid addition salts include inorganic acids such as, without limitation, sulfuric acid, phosphoric acid, hydrochloric acid, hydrobromic acid, hydriodic acid and the like, and organic acids, such as, without limitation, p-toluenesulfonic acid, methanesulfonic acid, oxalic acid, p-bromophenylsulfonic acid, carbonic acid, succinic acid, citric acid, benzoic acid, acetic acid, and the like.

Base addition salts include those derived from inorganic bases, such as, without limitation, ammonium hydroxide, alkaline metal hydroxide, alkaline earth metal hydroxides, carbonates, bicarbonates, and the like, and organic bases, such as, without limitation, ethanolamine, triethylamine, tris(hydroxymethyl)aminomethane, and the like. Examples of inorganic bases include sodium hydroxide, potassium hydroxide, potassium carbonate, sodium carbonate, sodium bicarbonate, potassium bicarbonate, calcium hydroxide, calcium carbonate, and the like.

The pharmaceutical composition of the present invention may be administered in oral forms, such as, without limitation normal and enteric coated tablets, capsules, pills, powders, granules, elixirs, tinctures, solution, suspensions, syrups, solid and liquid aerosols and emulsions. They may also be administered in parenteral forms, such as, without limitation, intravenous, intraperitoneal, subcutaneous, intramuscular, and the like forms, well-known to those of ordinary skill in the pharmaceutical arts. The pharmaceutical composition of the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using transdermal delivery systems well-known to those of ordinary skilled in the art.

The dosage regimen with the use of the pharmaceutical compositions of the present invention is selected by one of ordinary skill in the arts, in view of a variety of factors, including, without limitation, age, weight, sex, and medical condition of the recipient, the severity of the condition to be treated, the route of administration, the level of metabolic and excretory function of the recipient, the dosage form employed.

The pharmaceutical compositions of the present invention are preferably formulated prior to administration and include one or more pharmaceutically acceptable excipients. Excipients are inert substances such as, without limitation carriers, diluents, flavoring agents, sweeteners, lubricants, solubilizers, suspending agents, binders, tablet disintegrating agents and encapsulating material.

Yet in another embodiment, the pharmaceutical formulation of the present invention comprises one or more pharmaceutically-acceptable excipients that are compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. In making the compositions of the present invention, the active ingredient may be mixed with a diluent, or enclosed within a carrier, which may be in the form of a capsule, sachet, paper, or other container. The carrier may serve as a diluent, which may be solid, semi-solid, or liquid material which acts as a vehicle, or can be in the form of tablets, pills powders, lozenges, elixirs, suspensions, emulsions,

solutions, syrups, aerosols, ointments, containing, for example, up to 10% by weight of the active pharmaceutical composition, soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders.

For oral administration, the active ingredient may be combined with an oral, and non-toxic, pharmaceutically-acceptable carrier, such as, without limitation, lactose, starch, sucrose, glucose, sodium carbonate, mannitol, sorbitol, calcium carbonate, calcium phosphate, calcium sulfate, methyl cellulose, and the like; together with, optionally, disintegrating agents, such as, without limitation, maize, starch, methyl cellulose, agar bentonite, xanthan gum, alginic acid, and the like; and optionally, binding agents, for example, without limitation, gelatin, natural sugars, beta-lactose, corn sweeteners, natural and synthetic gums, acacia, tragacanth, sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes, and the like; and, optionally, lubricating agents, for example, without limitation, magnesium stearate, sodium stearate, stearic acid, sodium oleate, sodium benzoate, sodium acetate, sodium chloride, talc, and the like.

In powder forms, the carrier may be a finely divided solid which is in admixture with the finely divided active ingredient. The active ingredient may be mixed with a carrier having binding properties in suitable proportions and compacted in the shape and size desired to produce tablets. The powders and tablets preferably contain from about 1 to about 99 weight percent of the active ingredient which is the novel composition of the present invention. Suitable solid carriers are magnesium carboxy-methyl cellulose, low melting waxes, and cocoa butter.

Sterile liquid formulations include suspensions, emulsions, syrups and elixirs. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable carriers, such as sterile water, sterile organic solvent, or a mixture of both sterile water and sterile organic solvent.

The active ingredient can also be dissolved in a suitable organic solvent, for example, aqueous propylene glycol. Other compositions can be made by dispersing the finely divided active ingredient in aqueous starch or sodium carboxymethyl cellulose solution or in a suitable oil.

The formulation may be in unit dosage form, which is a physically discrete unit containing a unit dose, suitable for administration in human or other mammals. A unit dosage form can be a capsule or tablets, or a number of capsules or tablets. A "unit dose" is a predetermined quantity of the active pharmaceutical composition of the present invention, calculated to produce the desired therapeutic effect, in association with one or more excipients. The quantity of active ingredient in a unit dose may be varied or adjusted from about 0.1 to about 1000 milligrams or more according to the particular treatment involved.

Typical oral dosages of the present invention, when used for the indicated effects, will range from about 0.01mg /kg/day to about 100 mg/kg/day, preferably from 0.1 mg/kg/day to 30 mg/kg/day, and most preferably from about 0.5 mg/kg/day to about 10 mg/kg/day. In the case of parenteral administration, it has generally proven advantageous to administer quantities of about 0.001 to 100mg /kg/day, preferably from 0.01 mg/kg/day to 1 mg/kg/day. The pharmaceutical compositions of the present invention may be administered in a single daily dose, or the total daily dose may be administered in divided doses, two, three, or more times per day. Where delivery is via transdermal forms, of course, administration is continuous.

EXAMPLES

The present invention will be described as a form of examples, but they should by no means be construed as defining the metes and bounds of the present invention.

In the examples below, all quantitative data, if not stated otherwise, relate to percentages by weight.

The effect of the present pharmaceutical compositions was examined by the following assays and pharmacological tests.

EXAMPLE 1

[Measurement of capsaicin-induced Ca^{2+} influx in the human VR1-transfected CHO cell line] (Assay 1)

(1) Establishment of the human VR1-CHOluc9aeq cell line Human vanilloid receptor (hVR1) cDNA was cloned from libraries of axotomized dorsal root ganglia (Curtis, R. A. J., WO200029577). The cloned hVR1 cDNA was constructed with pcDNA3 vector and transfected into a CHOluc9aeq cell line, which contains aequorin and CRE-luciferase reporter genes as read-out signals. The transfectants were cloned by limiting dilution in selection medium (DMEM/F12 medium (Gibco BRL) supplemented with 10% fetal calf serum (FCS), 1.4 mM Sodium pyruvate, 20 mM HEPES, 0.15% Sodium bicarbonate, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM glutamine, non-essential amino acids and 2 mg/ml G418). Ca^{2+} influx was examined in the capsaicin-stimulated clones. A high responder clone was selected and used for further experiments. The human VR1-CHOluc9aeq cells were maintained in the selection medium in 5% CO_2 – air humidified atmosphere at 37 °C and passaged every 3-4 days at 1-2.5x10^5 cells/flask (75 mm^2).

(2) Measurement of Ca^{2+} influx using FDSS-3000

Human VR1-CHOluc9aeq cells were suspended in a culture medium which is the same as the selection medium except for G418 and seeded at a density of 1,000 cells per well into 384-well plates (black walled clear-base / Nalge Nunc International). Following the culture for 48 hrs the medium was changed to 2 μM Fluo-3 AM (Molecular Probes) and 0.02% Puronic F-127 in assay buffer (Hank's balanced salt solution (HBSS), 17 mM HEPES (pH7.4), 1 mM Probenecid, 0.1% bovine serum albumin (BSA)) and the cells were incubated for 60 min at 25°C. After washing twice with assay buffer the cells were incubated with a test compound or vehicle (dimethylsulphoxide) for 20 min at 25°C. The fluorescence changes indicating mobilization of cytoplasmic Ca^{2+} was measured by FDSS-3000 (Excitation wave-length at 488 nm, Emission wave-length at 540 nm / Hamamatsu Photonics) for 60 sec after the stimulation with 10 nM capsaicin. Integral R of the fluorescence changes was calculated in the samples treated with a test compound and vehicle respectively. Inhibitory effect of the compound was calculated by a comparison of the integral R values.

EXAMPLE 2

[Measurement of capsaicin-induced Ca^{2+} influx in the rat VR1-transfected CHO cell line] (Assay 2)

(1) Establishment of the rat VR1-CHOluc9aeq cell line

Rat vanilloid receptor (rVR1) cDNA was cloned from libraries of axotomized dorsal root ganglia (Caterina, M.J., et al, Nature, 389 (6653), 816-824 (1997)). The cloned rVR1 cDNA were constructed with pcDNA3 vector and transfected into the CHOluc9aeq cell line, which contains aequorin and CRE-luciferase reporter genes as read-out signals. The transfectants were cloned by limiting dilution in selection medium (DMEM/F12 medium (Gibco BRL)

supplemented with 10% fetal calf serum (FCS), 1.4 mM Sodium pyruvate, 20 mM HEPES, 0.15% Sodium bicarbonate, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, non-essential amino acids and 2 mg/ml G418). Ca^{2+} influx was examined in the capsaicin-stimulated clones. A high responder clone was selected and used for further experiments. The rat VR1-CHOluc9aeq cells were maintained in the selection medium in 5% CO_2 – air humidified atmosphere at 37 °C and passaged every 3-4 days at 1-2.5x10^5 cells/flask (75 mm^2).

(2) Measurement of Ca^{2+} influx using FDSS-3000

Rat VR1-CHOluc9aeq cells were suspended in a culture medium which is the same as the selection medium except for G418 and seeded at a density of 1,000 cells per well into 384-well plates (black walled clear-base / Nalge Nunc International). Following the culture for 48 hrs the medium was changed to 2 μM Fluo-3 AM (Molecular Probes) and 0.02% Puronic F-127 in assay buffer (Hank's balanced salt solution (HBSS), 17 mM HEPES (pH7.4), 1 mM Probenecid, 0.1% bovine serum albumin (BSA)) and the cells were incubated for 60 min at 25°C. After washing twice with assay buffer the cells were incubated with a test compound or vehicle (dimethylsulphoxide) for 20 min at 25°C. The fluorescence changes indicating mobilization of cytoplasmic Ca^{2+} was measured by FDSS-3000 (Excitation wave-length at 488nm, Emission wave-length at 540 nm / Hamamatsu Photonics) for 60 sec after the stimulation with 10 nM capsaicin. Integral R of the fluorescence changes was calculated in the samples treated with a test compound and vehicle respectively. Inhibitory effect of the compound was calculated by a comparison of the integral R values.

EXAMPLE 3

[Measurement of the capsaicin-induced Ca^{2+} influx in primary cultured rat dorsal root ganglia neurons] (Assay 3)

(1) Preparation of rat dorsal root ganglia neurons

New born Wister rats (5-11 days) were sacrificed and dorsal root ganglia (DRG) was removed. DRG was incubated with 0.1% trypsin (Gibco BRL) in phosphate balanced solution (PBS)(-) (Gibco BRL) for 30 min at 37°C, then a half volume of fetal calf serum (FCS) was added and the cells were spun down. The DRG neuron cells were resuspended in Ham F12/5% FCS/5% horse serum (Gibco BRL) and dispersed by repeated pipetting and passing through 70 µm mesh (Falcon) and seeded into a 96-well culture plate. The culture plate was incubated for 3 hours at 37°C to remove contaminating Schwann cells. Non-adherent cells were recovered and further cultured in laminin-coated 384 well plates (Nunc) at a density of $1x10^4$ cells/50 µl/well for 2 days in the presence of 50 ng/ml recombinant rat NGF (Sigma) and 50 µM 5-fluorodeoxyuridine (Sigma).

(2) Ca^{2+} mobilization assay

DRG neuron cells were washed twice with assay buffer (HBSS supplemented with 17 mM HEPES (pH 7.4) and 0.1% BSA). After incubating with 2 µM fluo-3AM (Molecular Probe), 0.02% PF127 (Gibco BRL) and 1 mM probenecid (Sigma) for 40 min at 37°C, and cells were washed with assay buffer 3 times. The cells were incubated with VR1 antagonists or vehicle (di-methylsulphoxide) and then with 1 µM capsaicin in FDSS-6000 (Excitation wave-length at 480nm, Emission wave-length at 520nm / Hamamatsu Photonics). The fluorescence changes at 520nm were monitored for 2.5 min. Integral R of the fluorescence change was calculated in the samples treated with a compound and vehicle, respectively. Inhibitory effect of the compound was calculated by comparison of the integral R values.

EXAMPLE 4

[Organ bath assay to measure the capsaicin-induced bladder contraction] (Assay 4)

Male Wistar rats (10 week old) were anesthetized with ether and sacrificed by dislocating the necks. The whole urinary bladder was excised and placed in Modified Krebs-Henseleit solution (pH 7.4) of the following composition (112mM NaCl, 5.9mM KCl, 1.2mM $MgCl_2$, 1.2mM NaH_2PO_4, 2mM $CaCl_2$, 2.5mM $NaHCO_3$, 12mM glucose). The solution was saturated with 95% O_2 and 5% CO_2 mixture at 37°C and pH7.4. Contractile responses of the urinary bladder were studied as described previously (Maggi CA et al, Br.J.Pharmacol. 108: 801-805, 1993). Isometric tension was recorded under a load of 1 g using longitudinal strips of rat detrusor muscle. Bladder strips were equilibrated for 60 min before each stimulation. Contractile response to 80 mM KCl was determined at 15 min intervals until reproducible responses were obtained. The response to KCl was used as an internal standard to evaluate the maximal response to capsaicin. The effects of the compounds were investigated by incubating the strips with compounds for 30 min prior to the stimulation with 1 μM capsaicin (vehicle: 80% saline, 10% EtOH, and 10% Tween 80). One of the preparations made from the same animal was served as a control while the others were used for evaluating compounds. Ratio of each capsaicin-induced contraction to the internal standard (i.e. KCl-induced contraction) was calculated and the effects of the test compounds on the capsaicin-induced contraction were evaluated.

EXAMPLE 5

[Measurement of capsaicin-induced over active bladder in anesthetized rats] (Assay 5)

(1) Animals

Female Sprague-Dawley rats (180~250 g / Charles River Japan) were used.

(2) Catheter implantation

Rats were anesthetized by intraperitoneal administration of urethane (Sigma) at 1.25 g/kg. The abdomen was opened through a midline incision, and a polyethylene catheter (BECTON DICKINSON, PE50) was implanted into the bladder through the dome. In parallel, the inguinal region was incised, and a polyethylene catheter (BECTON DICKINSON, PE50) filled with saline (Otsuka) was inserted into a femoral vein.

(3) Cystometric investigation

The bladder catheter was connected via T-tube to a pressure transducer (Viggo-Spectramed Pte Ltd, DT-XXAD) and a microinjection pump (TERUMO). Saline was infused at room temperature into the bladder at a rate of 3.6 ml/hr. Intravesical pressure was recorded continuously on a chart pen recorder (Yokogawa). At least three reproducible micturition cycles, corresponding to a 20-minute period, were recorded before a capsaicin infusion and used as baseline values.

(4) Administration of test compounds and stimulation of bladder with capsaicin

A testing compound dissolved in the mixture of ethanol, Tween 80 (ICN Biomedicals Inc.) and saline (1 : 1 : 8, v/v/v) was administered intravenously at 3 mg/kg or 10 mg/kg. 2min after the administration of the compound, saline including 30 μM of capsaicin (Nacalai Tesque) was infused at room temperature into the bladder at a rate of 3.6 ml/hr.

(5) Analysis of cystometry parameters

The capsaicin-induced increases of micturition frequency were analyzed from the cystometry data. The testing compounds–mediated inhibition of the increased frequency was evaluated using unpaired Student's t-test. A probability levels less than 5% was accepted as significant difference.

EXAMPLE 6

[Measurement of over active bladder in anesthetized cystitis rats] (Assay 6)

(1) Animals

Female Sprague-Dawley rats (180~250 g / Charles River Japan) were used. Cyclophosphamide (CYP) dissolved in saline was administered intraperitoneally at 150 mg/kg 48 hours before experiment.

(2) Catheter implantation

Rats were anesthetized by intraperitoneal administration of urethane (Sigma) at 1.25 g/kg. The abdomen was opened through a midline incision, and a polyethylene catheter (BECTON DICKINSON, PE50) was implanted into the bladder through the dome. In parallel, the inguinal region was incised, and a polyethylene catheter (BECTON DICKINSON, PE50) filled with saline (Otsuka) was inserted into a femoral vein. After the bladder was emptied, the rats were left for 1 hour for recovery from the operation.

(3) Cystometric investigation

The bladder catheter was connected via T-tube to a pressure transducer (Viggo-Spectramed Pte Ltd, DT-XXAD) and a microinjection pump (TERUMO). Saline was infused at room temperature into the bladder at a rate of 3.6 ml/hr for 20 min. Intravesical pressure was recorded continuously on a chart pen recorder (Yokogawa). At least three reproducible micturition cycles, corresponding to a 20-minute period, were recorded before a test compound administration.

(4) Administration of test compounds

A testing compound dissolved in the mixture of ethanol, Tween 80 (ICN Biomedicals Inc.) and saline (1 : 1 : 8, v/v/v) was administered intravenously at 0.05 mg/kg, 0.5 mg/kg or 5 mg/kg. 3min after the administration of the compound, saline (Nacalai Tesque) was infused at room temperature into the bladder at a rate of 3.6 ml/hr.

(5) Analysis of cystometry parameters

The cystometry parameters were analyzed as described previously [Lecci A et al, Eur. J. Pharmacol. 259: 129-135, 1994]. The micturition frequency calculated from micturition interval and the bladder capacity calculated from a volume of infused saline until the first micturition were analyzed from the cystometry data. The testing compounds–mediated inhibition of the frequency and the testing compounds–mediated increase of bladder capacity were evaluated using unpaired Student's t-test. A probability levels less than 5% was accepted as significant difference. Data were analyzed as the mean $\pm$ SEM from 4 – 7 rats.

SELECTIVITY TEST

[Measurement of Ca^{2+} influx in the human P2X1-transfected CHO cell line]

(1) Preparation of the human P2X1-transfected CHOluc9aeq cell line

Human P2X1-transfected CHOluc9aeq cell line was established and maintained in Dulbecco's modified Eagle's medium (DMEM/F12) supplemented with 7.5% FCS, 20 mM HEPES-KOH (pH 7.4), 1.4 mM sodium pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine (Gibco BRL) and 0.5 Units/ml apyrase (grade I, Sigma). The suspended cells were seeded in each well of 384-well optical bottom black plates (Nalge Nunc

International) at 3 x 10^3 / 50 µl / well. The cells were cultured for following 48 hrs to adhere to the plates.

(2) Measurement of the intracellular Ca^{2+} levels

P2X1 receptor agonist-mediated increases in cytosolic Ca^{2+} levels were measured using a fluorescent Ca^{2+} chelating dye, Fluo-3 AM (Molecular Probes). The plate-attached cells were washed twice with washing buffer (HBSS, 17 mM HEPES-KOH (pH 7.4), 0.1% BSA and 0.5 units/ml apyrase), and incubated in 40 µl of loading buffer (1 µM Fluo-3 AM, 1 mM probenecid, 1 µM cyclosporin A, 0.01% pluronic (Molecular Probes)in washing buffer) for 1 hour in a dark place. The plates were washed twice with 40 µl washing buffer and 35 µl of washing buffer were added in each well with 5 µl of test compounds or *2',3'-o*-(2,4,6-trinitrophenyl) adenosine 5'-triphpsphate (Molecular Probes) as a reference. After further incubation for 10 minutes in dark 200 nM α, β-methylene ATP agonist was added to initiate the Ca^{2+} mobilization. Fluorescence intensity was measured by FDSS-6000 (λ_{ex}=410nm, λ_{em}=510nm / Hamamatsu Photonics) at 250 msec intervals. Integral ratios were calculated from the data and compared with that of a control.

The compounds were examined in the above-identified assays 1 to 5 and selectivity test. The results of 50% inhibitory concentration (IC_{50})and % inhibition are shown in table 1 below.

Table 1

	Assay 1	Assay 2	Assay 3	Assay 4	Selectivity Test	Assay 5
	Human VR1	RatVR1	Rat DRG Ca	Organ bath	Human P2X1	In Vivo Cystometry
	IC50 (nM)	IC50 (nM)	IC50 (nM)	IC50 (uM)	IC50 (nM)	%Inh i.v.
N-[4-chloro-3-(trifluoromethyl)phenyl]-N'-(2,4-dichloro-7-hydroxy-1-naphthyl)urea	5,9	2,1	45	20	7.100	55% @10mg/kg
N-(4-bromo-2-chloro-7-hydroxy-1-naphthyl)-N'-[4-chloro-3-(trifluoromethyl)phenyl]urea	6,8	4,9	270	2,5	4.700	88% @3mg/kg
N-[4-chloro-3-(trifluoromethyl)phenyl]-N'-(2,4-dibromo-7-hydroxy-1-naphthyl)urea	7,5	16	62	5,0	> 10.000	11% @3mg/kg 65% @10mg/kg

***In vitro* profile of VR1 antagonists (Assays 1 to 4 and selectivity test)**

As summarized in the table, the tested compounds inhibited the capsaicin-induced increase of intracellular calcium levels (Ca^{2+} flux) in the cell line expressing human and rat VR1 in a concentration dependent manner with IC_{50} values at nanomolar range. Functional activity (Ca^{2+} flux) in the capsaicin-stimulated rat DRG cells was inhibited by the tested compounds. Significant inhibition of the capsaicin-induced rat bladder detrusor contraction was observed for the tested compounds.

Selectivity over other ion channel receptors such as P2X1 was high – more than 100 fold.

***In vivo* profile of VR1 antagonists (Assays 5 and 6)**

The effect of VR1 antagonist on the capsaicin-induced overactive bladder *in vivo* in anesthetized rats was investigated. The overactive bladder was induced by intravesical infusion of capsaicin solution. The frequency of the micturition was compared.

Intravenous administration of VR1 antagonist inhibited the capsaicin-induced increase of micturition reflex at 3 or 10 mg/kg. (table 1).

As disclosed in assay 6, the effect of N-[4-chloro-3-(trifluoromethyl)phenyl]-N'-(2,4-dichloro-7-hydroxy-1-naphthyl)urea on cyclophosamide induced cystitis in anesthetized rats was investigated. Significant improvement of both bladder capacity (Fig. 1 and Fig. 2) and micturition frequency (Fig. 1 and Fig. 3) was observed at a dosage of 0.5 mg/kg, i.v. and 5 mg/kg, i.v. for N-[4-chloro-3-(trifluoromethyl)phenyl]-N'-(2,4-dichloro-7-hydroxy-1-naphthyl)urea.

Similar degree of the improvement was observed in the cyclophosamide induced cystitis in anesthetized rats, in which sensory nerve was desensitized by treatment with capsaicin (Fig. 1).

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 presents charts showing bladder capacity and voiding frequency in normal rats (Normal), cyclophosphamide treated rats (CYP-rats), CYP-rats treated with VR1 antagonist, (N-[4-chloro-3-(trifluoromethyl)phenyl]-N'-(2,4-dichloro-7-hydroxy-1-naphthyl)urea) (CYP-VR1) and CYP-rats treated with capsaicin to desensitize sensory nerve (CYP-capsaicin).

Fig. 2 presents graphs which show the bladder capacity in normal rats (Normal), cyclophosphamide treated rats (CYP-rats) and CYP-rats treated with VR1 antagonist (N-[4-chloro-3-(trifluoromethyl)phenyl]-N'-(2,4-dichloro-7-hydroxy-1-naphthyl)urea) (CYP-VR1).

Fig. 3 presents graphs which show the micturition frequency in normal rats (Normal), cyclophosphamide treated rats(CYP-rats) and CYP-rats treated with VR1 antagonist (N-[4-chloro-3-(trifluoromethyl)phenyl]-N'-(2,4-dichloro-7-hydroxy-1-naphthyl)urea) (CYP-VR1).

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Claims

(1) A method for treating urological disorder in a mammal, said method comprising administering to said mammal a therapeutically effective amount of a vanilloid receptor (VR1) antagonist, or a pharmaceutically acceptable salt thereof, or pharmaceutical composition containing either entity.

(2) The method of claim 1, wherein said urological disorder is overactive bladder.

(3) The method of claim 2, wherein said overactive bladder includes detrusor hyper-reflexia, detrusor instability, urgenct micturition and micturition of increased frequency.

(4) The method of claim 1, wherein said urological disorder is urinary incontinence.

(5) The method of claim 4, wherein said urinary incontinence includes urge urinary incontinence and mix urinary incontinence.

(6) The method of claim 1, wherein said mammal is a human.

(7) The method of claim 1, wherein said VR1 antagonist is selected from the group consisting of:
N-[4-chloro-3-(trifluoromethyl)phenyl]-N'-(2,4-dibromo-7-hydroxy-1-naphthyl)urea;
N-[4-chloro-3-(trifluoromethyl)phenyl]-N'-(2,4-dichloro-7-hydroxy-1-naphthyl)urea and
N-(4-bromo-2-chloro-7-hydroxy-1-naphthyl)-N'-[4-chloro-3-(trifluoromethyl)phenyl]urea,
Or pharmaceutically acceptable salts thereof.

(8) The method of claim 1, wherein said VR1 antagonist is an antibody immunoreactive with VR1.

(9) The method of claim 1, wherein said VR1 antagonist is administered in combination with a pharmaceutically acceptable carrier.

(10) Use of a VR1 antagonist in the preparation of a medicament for treating or preventing urological disorder.

(11) The use of claim 10, wherein said urological disorder is overactive bladder.

(12) The use of claim 11, wherein said overactive bladder includes detrusor hyper-reflexia, detrusor instability, urgenct micturition and micturition of increased frequency.

(13) The use of claim 12, wherein said urological disorder is urinary incontinence.

(14) The use of claim 13, wherein said urinary incontinence includes urge urinary incontinence and mix urinary incontinence.

(15) The use of claim 10, wherein said VR1 antagonist is selected from the group consisting of

N-[4-chloro-3-(trifluoromethyl)phenyl]-N'-(2,4-dibromo-7-hydroxy-1-naphthyl)urea;

N-[4-chloro-3-(trifluoromethyl)phenyl]-N'-(2,4-dichloro-7-hydroxy-1-naphthyl)urea and

N-(4-bromo-2-chloro-7-hydroxy-1-naphthyl)-N'-[4-chloro-3-(trifluoromethyl)phenyl]urea,

Or pharmaceutically acceptable salts thereof.

(16) The use of claim 10, wherein said VR1 antagonist is an antibody immunoreactive with VR1.

(17) A pharmaceutical composition comprising a VR1 antagonist for treatment and/or prophylaxis of urological disorder.

(18) The pharmaceutical composition of claim 17, wherein said urological disorder is overactive bladder.

(19) The pharmaceutical composition of claim 18, wherein said overactive bladder includes detrusor hyper-reflexia, detrusor instability, urgenct micturition and micturition of increased frequency.

(20) The pharmaceutical composition of claim 17, wherein said urological disorder is urinary incontinence.

(21) The pharmaceutical composition of claim 20, wherein said urinary incontinence includes urge urinary incontinence and mix urinary incontinence.

(22) The pharmaceutical composition of claim 17, wherein said VR1 antagonist is selected from the group consisting of

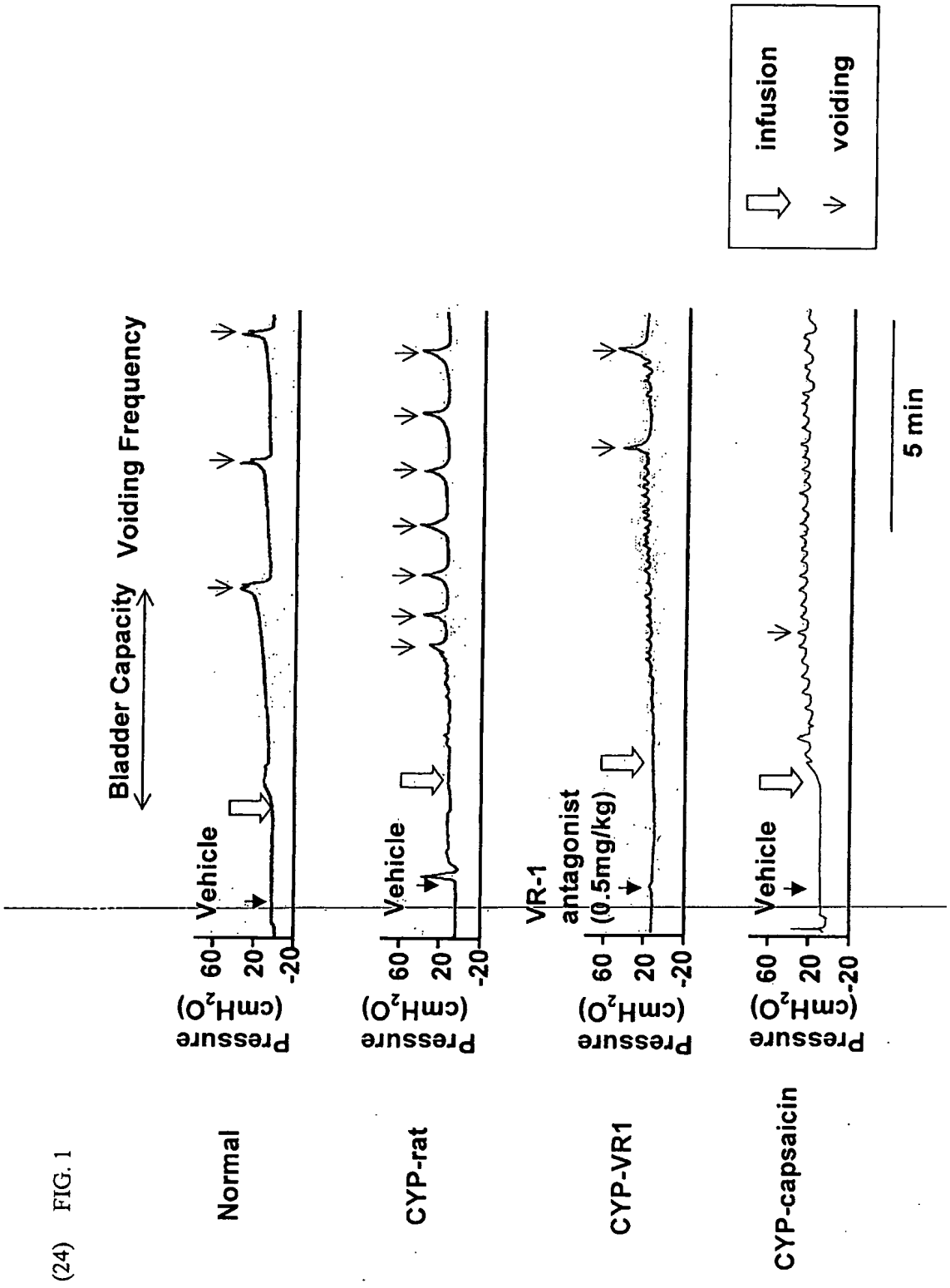
N-[4-chloro-3-(trifluoromethyl)phenyl]-N'-(2,4-dibromo-7-hydroxy-1-naphthyl)urea;

N-[4-chloro-3-(trifluoromethyl)phenyl]-N'-(2,4-dichloro-7-hydroxy-1-naphthyl)urea and

N-(4-bromo-2-chloro-7-hydroxy-1-naphthyl)-N'-[4-chloro-3-(trifluoromethyl)phenyl]urea,

Or pharmaceutically acceptable salts thereof.

(23) The pharmaceutical composition of claim 17, wherein said VR1 antagonist is an antibody immunoreactive with VR1.


Bladder Capacity
Voiding Frequency
infusion
voiding
Normal
CYP-rat
CYP-VR1
CYP-capsaicin
Vehicle
Vehicle
VR-1 antagonist (0.5mg/kg)
Vehicle
Pressure (cmH2O)
60
20
-20
5 min

(24) FIG. 1

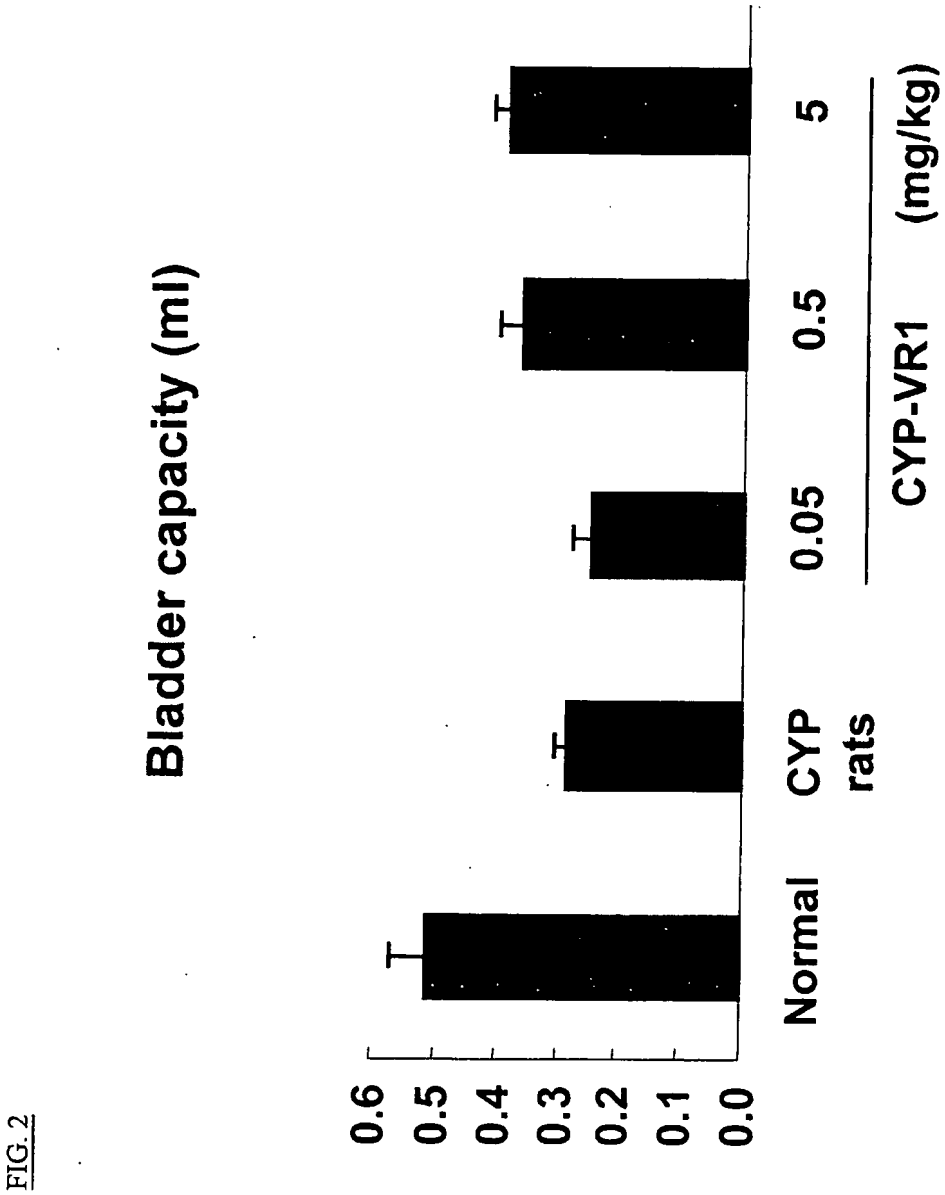

Bladder capacity (ml)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Normal
CYP rats
0.05
0.5
5
CYP-VR1 (mg/kg)

FIG. 2

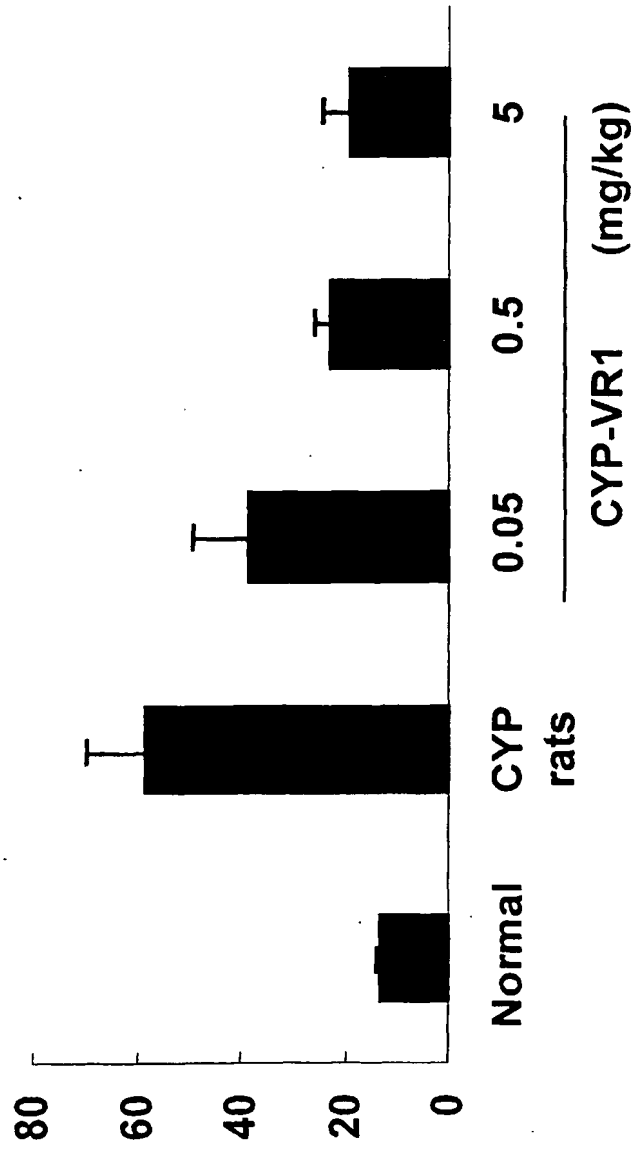

Voiding frequency
(no. of voiding / hr)
80
60
40
20
0
Normal
CYP
rats
0.05
0.5
5
CYP-VR1 (mg/kg)

FIG.3